A newly generated, high quality DNA reference database for European fish species reveals substitution fraud of processed Atlantic cod (*Gadus morhua*) and common sole (*Solea solea*) at different steps of the Belgian supply chain

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Seafood forms an important part of the human diet, but fish stocks are under pressure globally. This leads to fraudulent practices such as substitution of higher value species with cheaper alternatives. Substitution is more likely to occur in processed seafood products, as species can no longer be identified visually. Molecular techniques, such as DNA barcoding by means of mitochondrial markers, do allow for a proper identification of processed food by comparing a small DNA fragment to a reference database. However, public reference sequence databases contain sequences linked to misleading species names, thereby hampering accurate identification of the sequences.

A reliable high quality database for COI and Cytb sequences (and rhodopsin for hybrids) of commercially important fish species in Europe has been created to ensure correct identification through DNA barcoding. Specimens from the Baltic sea, inland waters of North East Europe, the Northeast Pacific, the North Sea and, Northeast Atlantic Ocean, next to specimens from aquaculture, were collected. In total, 300 sequences (145 COI, 152 Cytb and 3 Rhodopsin genes) for 42 economically important fish species were generated using universal primers and Sanger sequencing.

Other studies already showed that fish species are often substituted by cheaper species at the end of the food supply chain, more specifically, in restaurants, canteens and food services. Little is known about the prevalence of substitution in other parts of the supply chain, although a 'knock-on-effect' can be anticipated. To gain insight in the current situation of seafood trade in the Belgian fish supply chain, we performed interviews with local stakeholders and scientists, topped with information from reports and literature. We identified nine steps where morphologically unrecognisable fish are potentially traded: wholesale, import, retailers, fishmongers, processing, the fishermen's market, catering, food services and export.

To assess how substitution evolves throughout the Belgian supply chain, we collected morphologically unrecognisable food product samples of two commercially important species: Atlantic cod, *Gadus morhua,* and common sole, *Solea solea,* at different steps of the supply chain. DNA barcoding of the COI and Cytb gene was used to identify both *G. morhua* and *S. solea* samples. Additionally, a species specific qPCR assay was used to identify *G. morhua*. Of the 138 cod samples, only 3 were substituted, situated in catering (6 %), import (5 %) and fishmongers (3 %). Of the 45 sole samples, 7 samples were substituted, situated in wholesale (100 %), food

services (50 %), retailers (20 %) and catering (8 %). Substitutes for *G. morhua* were morphologically similar Gadidae species, such as *G. chalcogrammus, Pollachius virens* and *Melanogrammus aeglefinus.* The substitutes for *S. solea* were morphologically similar flatfish and Soleidae species, being *S. senegalensis, Limanda aspera, Lepidopsetta polyxstra, Cynoglossus sp. and Microstomus kitt*, with one exception being *Pangasianodon hypophthalmus.* In conclusion, the newly generated genetic reference database proved to be a useful tool for the identification of processed samples. Substitution in the Belgian food supply chain occurs for *S. solea* and to a lesser extent for *G. morhua,* not only in restaurants, but also in other parts of the supply chain. *L. aspera* seems to be an increasingly popular alternative to *S. solea* due to its lower price. More stringent control measures along the complete supply chain are required to ensure more transparency, safety and trust to allow Belgian consumers to trust their purchases.

Keywords: Fish fraud; Substitution; Belgian food supply chain; Atlantic cod; Common sole; DNA barcoding; COI; Cytb; qPCR; Adulteration